REMARKS

Claim 1 has been amended to clarify that the agent with the Markush group is the differentiation agent. Support for this amendment can be found in claim 1 as originally filed. Claim 1 has further been amended to clarify that the pine is of the subgenus *Pinus*, the subgenus to which Southern yellow pines belong. Finally, claim 1 has been amended to clarify that it is the selected transgenic embryogenic cells that are regenerated into genetically modified plants.

It is submitted that these amendments do not constitute new matter and their entry is requested.

The Examiner has rejected claims 1-3, 5-8, 10, 11, 14-17, 19, 20 and 22-43 under 35 U.S.C. §103(a) as being obvious over Wenck et al. (*Plant Molecular Biology* 39:407-416, 1999) taken with Rutter et al. (US 5,731,204) and Levee et al. (*Molecular Breeding* 5:429-440, 1999). It is submitted that the claimed invention is not obvious from the teachings of the cited art.

In accordance with the claimed invention, genetically modified plants of pine of the subgenus *Pinus* are regenerated by selecting transgenic embryogenic pine cells using a selection medium comprising a selection agent and a differentiation agent and then regenerating plants from the selected transgenic embryogenic pines cells. The pine of the subgenus *Pinus* are selected from the group consisting of Southern yellow pines and hybrids thereof. The differentiation agent is selected from the group consisting of abscisic acid (ABA), polytheylene glycol (PEG), a gelling agent in an amount between about 3% and about 5% or between about 0.5% and about 1.5% and combinations thereof. Applicants discovered that the presence of the differentiation agent in the selection medium allowed for the first time the regeneration of transgenic plants of pine of the subgenus *Pinus*, especially Southern yellow pines and particularly lines of certain elite genetic backgrounds of the Southern yellow pines.

At page 3 of the Office Action, the Examiner states,

> Wenck et al. teach a method of genetically transforming Norway spruce using *Agrobacterium* and selecting the transformed embryogenic lines using kanamycin as an agent to select transformants and incorporating into the media abscisic acid which acts as an agent to regulate differentiation of embryos from embryogenic

> cells (page 409, left column, first line and right column, 'Embryo maturation' paragraph, respectively.)

However, Wenck et al. does not contain any such disclosure. Wenck et al. does disclose the use of a selection agent, i.e., kanamycin, in the selection medium. The selection medium is 471 medium. This medium **does not contain ABA** as shown by the medium components in Table 2 on page 410. Thus, transformed cells are selected on a medium that **does not contain ABA**. "Selected" cell lines are then cultured to produce mature embryos. The culturing of the "selected" cell lines is done on medium that contains ABA. It is the production of mature embryos from selected cells that is done on medium containing ABA **not** the selection of transgenic embryogenic cells on a selection medium that contains ABA. Consequently, not only does Wenck et al. not teach transforming and regenerating genetically modified Southern yellow pine plants (in fact, it teaches that it was unable to transform and regenerate genetically modified Southern yellow pine plants; *see* page 413, left column), but it also does not teach using ABA in the selection medium.

At page 7 of the Office Action, the Examiner states,

> The Office contends, that absent evidence to the contrary, the method of Wenck et al. of transforming and regenerating one conifer, Norway spruce, can be applied to transforming and regenerating another conifer, that being Southern yellow pines.

Applicants note that Wenck et al., itself contains evidence to the contrary. Wenck et al. discloses experiments for the transformation and regeneration of not only Norway spruce, but also loblolly pine, a Southern yellow pine. Wenck et al. report the successful regeneration of transformed cells of Norway spruce. However, Wenck et al. report the unsuccessful regeneration of transformed loblolly pine. *See* page 413, left column. Thus, the very art cited by the Examiner contains evidence that the method of Wenck et al. for transforming and regenerating transformed plants of one conifer is not applicable to transforming and regenerating another conifer.

Neither of the secondary references disclose the use of a differentiation agent as specified in the claims in a selection medium. There is no disclosure of transgenic embryogenic pine cells of Southern yellow pines in Rutter et al., and consequently, there is no disclosure of a selection medium. Since there is no disclosure of a selection medium in Rutter et al., there is no disclosure

of the use of any one of the differentiation agents specified in the claims in a selection medium. Thus, Rutter et al. does not disclose these elements of the claimed invention. Similarly, Levee et al. does not disclose the use of any one of the differentiation agents specified in the claims in a selection medium for selecting transgenic tissue. Furthermore, Levee et al. does not describe the regeneration of genetically modified pine plants of the subgenus *Pinus*. Thus, Levee et al. does not disclose these elements of the claimed invention. Since none of the cited references disclose the use of the differentiation agents specified in the claims in a selection medium and do not disclose the regeneration of genetically modified Southern yellow pines, the cited references do not and cannot render the claimed invention obvious. On this basis alone, Applicants submit that the Examiner should withdraw the obviousness rejection.

Furthermore, Applicants have previously noted differences between hard and soft pines and the prior inability to recover selected cells at a level which would be commercially useful for producing transgenic pine, especially for Southern yellow pines and hybrids thereof, which are hard pines. As discussed above, one feature of the invention which enabled the recovery of transgenic cells for regenerating transgenic plants was the use of a differentiation agent in the selection medium. The use of the differentiation agent was in addition to the selection agent which is used to select transgenic cells. The use of the differentiation agent ensured that transgenic cells could be recovered following selection and these transgenic cells could be regenerated into transgenic plants in commercially useful quantities. For example, prior art techniques resulted in a 26% rate of regeneration for loblolly pine (*Pinus taeda*), whereas the present invention resulted in a 71% rate of regeneration. Similarly, for elite families, the prior art was unable to achieve regeneration, whereas the present invention achieved an 80% rate of regeneration. In the previous Amendment, Applicants discussed the known differences between hard and soft pines and the unobvious nature of plant transformation and regeneration in these species. These differences present additional evidence that transformation and regeneration in one conifer could not be applied to another conifer.

It is well known in the art that white pine (such as disclosed in Levee et al.) is a soft pine of the subgenus *Strobus* and not a hard pine of the subgenus *Pinus*, such as the Southern yellow pines

and hybrids thereof. For example, most classifications of *Pinus* recognize two major lineages: subgenus *Strobus* (haploxylon or soft pines, with one fibrovascular bundle in the needle) and subgenus *Pinus* (diploxylon or hard pines, with two fibrovascular bundles in the needle). This division is consistent with data from wood anatomy and secondary chemistry, and is supported in recent molecular phylogenetic studies (Strauss and Doerksen, 1990, *Evolution* **44**:1081-1096; Wang and Szmidt, 1993, *Plant Systematics and Evolution* **188**:197-211; reviewed in Price et al., 1998, in *Ecology and Biogeography of Pinus*, Cambridge University Press, Cambridge, pp. 49-68).

Pines have a relatively rich fossil record dating back to the Early Cretaceous, 130 million years ago (review in Axelrod et al., 1986, *Ann Mo Bot Gard* **73**:565-641; Klaus et al., 1989, *Plant Systematics and Evolution* **162**:133-163; Van der Burgh, 1973, *Review of Paleobotany and Palynology*, **15**:73-275; Millar, 1993, *Ann Mo Bot Gard* **80**:471-498). The genetic distance between subgenera, at least between *Pinus* and *Strobus*, may be as large as, or larger than the genetic distance between other conifer genera, e.g., between *Cedrus* and *Abies* (Price et al., 1987, *Systematic Botany*, **12**:91-97), and if strict genetic criteria were used, they should perhaps be treated at generic rank. As is commonly known, hard pines are unable to breed with soft pines, though they can interbreed readily, if the correct timing and other conditions are provided, with other hard pine species (a seminal reference is Critchfield and Little, 1966, *Geographic distribution of the pines of the world*, USDA Forest Service Miscellaneous Publication 991, Washington, D.C.; see also Little and Critchfield, 1969, *Subdivision of the genus Pinus pines*, USDA Forest Service Miscellaneous Publication 1144, Washington, D.C.). Hard pines are unaffected by a number of diseases, such as white pine blister rust, that readily infect soft pines. Their susceptibility to *Agrobacterium* infection appears to be quite different as well.

As previously noted, Levee et al., the only cited prior art that discloses regeneration of a transformed pine, discloses the transformation and regeneration of pine of the subgenus *Strobus* which, according to this reference, "is the first work on genetic transformation on **this pine species** as well as the first report of successful stable genetic transformation of **a pine species** using a disarmed strain of *A. tumefaciens*". (See page 36, first paragraph of Discussion, emphasis added).

Levee et al. does not disclose the transformation and regeneration of pine of the subgenus *Pinus*. The amended claims are clearly directed to pine cells of the *Pinus* genus. It is well known to those skilled in the art that somatic embryogenesis systems for soft pines are different from those for hard pines, such as Southern yellow pines and hybrids thereof. It is not insignificant that Levee et al. utilized a soft pine which is more easily regenerated than hard pines. Although the Examiner cited art showing transformation and regeneration of soft pine, he has not cited any art showing transformation and regeneration of hard pines as set forth in the claims. Furthermore, it is submitted that there have been no reports in the literature of the regeneration of plants following stable transformation of embryogenic cultures of any pines of the *Pinus* subgenus by *Agrobacterium*.

Applicants have previously discussed differences between hard and soft pines and the prior inability to regenerate transformed pine tissue of pines of the subgenus *Pinus*, i.e., hard pines, in commercially valuable quantities. One feature of the invention which enables the enhanced transformation and the regeneration of transformed embryonic hard pine tissue is the use of a liquid wash medium as opposed to the use of water to wash cells following *Agrobacterium* infection or cocultivation of embryonic hard pine tissue with *Agrobacterium*. This feature of the invention is found in the claims. The differences between hard and soft pines leads to the unobvious nature of plant transformation and regeneration in these species. In accordance with the Examiner's earlier suggestion that Declarations be submitted to address this point, Applicants are submitting concurrently herewith Rule 132 Declarations of Dr. Marie B. Connett-Porceddu and Dr. Michael R. Becwar. In addition, Applicants are submitting copies of the Rule 132 Declarations of Mr. David S. Canavera and Dr. James E. Mann that were filed in companion application Serial No. 09/973,088.

The Declaration Under Rule 132 of Marie B. Connett-Porceddu (hereinafter the "Connett Declaration") describes the nonobvious nature of the present invention over the art cited by the Examiner. Specifically, Dr. Connett-Porceddu provides a comparison of the regeneration frequencies for transgenic Southern yellow pines and hybrids thereof using (a) standard selection conditions of the prior art for selection and (b) selection conditions of the present invention, i.e., the use of a differentiation agent as specified in the claims in the selection medium along with a

selection agent. *See* Paragraph 10 of the Connett Declaration. According to this comparison, the regeneration frequency for transgenic *P. taeda* using the standard selection conditions was 26%, whereas the regeneration frequency for transgenic *P. taeda* using the present invention was 71%. The regeneration frequency for transgenic *P. taeda* elite families using the standard selection conditions was 0%, whereas the regeneration frequency for transgenic *P. taeda* elite families using the present invention was 80%. *See* Paragraph 10 of the Connett Declaration.

Dr. Connett-Porceddu discusses the teachings of the prior art cited by the Examiner. She states that Wenck et al. specifically teaches that genetically modified plants of Southern yellow pines had not been obtained. *See* Paragraph 11 of the Connett Declaration. This result is opposite to the result that Wenck et al. achieved for Norway spruce, a different conifer.

Dr. Connett-Porceddu states that the prior art method of Rutter et al. of using PEG and ABA in the regeneration of pine plants from tissue culture did not result in the germination somatic embryos. *See* Paragraph 12 of the Connett Declaration. She also states that Rutter et al. does not describe a method (a) for the selection of genetically modified pine cells, i.e., the selection of transgenic embryogenic pine cells of Southern yellow pines or hybrids thereof or (b) for the regeneration of genetically modified plants from selected transgenic pine cells. *See* Paragraph 12 of the Connett Declaration. In view of this lack of teachings in Rutter et al., it is Dr. Connett-Pordeddu's opinion that a skilled artisan would have no expectation of success for modifying the method of Wenck et al. to regenerate genetically modified plants of Southern yellow pines. *See* Paragraph 12 of the Connett Declaration.

Dr. Connett-Porceddu states that the Levee et al. prior art discloses the transformation and regeneration of a soft pine (i.e., a pine of the subgenus *Strobus*) and does not disclose the transformation and regeneration of a hard pine (i.e., a pine of the subgenus *Pinus* such as a Southern yellow pine). *See* Paragraph 13 of the Connett Declaration. She also states that Levee does not teach the use of a differentiation agent as specified in the claims in the selection medium with a selection agent for selecting transgenic pine cells of Southern yellow pines. *See* Paragraph 13 of the Connett Declaration. Finally, Dr. Connett-Porceddu states that a skilled artisan would expect that the method

of Levee et al. for soft pines could be used or routinely modified for use with hard pines. *See* Paragraph 13 of the Connett Declaration.

Dr. Connett-Porceddu states that it was well known that there were differences between soft pines and hard pines. These differences were seen in the transformation and regeneration of hard and soft pines, such that there was no expectation of success concerning the transformation and regeneration of hard pines or soft pines on the basis of the other. *See* Paragraph 14 of the Connett Declaration. This knowledge and lack of expectation of success is described in detail in Exhibit 2 to the Connett Declaration and in the Declaration Under Rule 132 of Michael R. Becwar, each of which are discussed in further detail below.

In view of these facts, Dr. Connett-Porceddu concludes that a skilled artisan would have no expectation of success for modifying the method of Wenck et al. to regenerate genetically modified plants of Southern yellow pines or hybrids thereof. *See* Paragraphs 15-17 of the Connett Declaration.

Exhibit 2 to the Declaration Under Rule 132 of Marie B. Connett-Porceddu is a copy of the Rule 132 Declaration of Dr. Marie B. Connett-Porceddu submitted in companion application Serial No. 09/973,088 (hereinafter the "Exhibit 2 Connett Declaration") that describes the known differences between hard pines and soft pines and the unobviousness of the method claimed in the present application. Specifically, Dr. Connett-Porceddu states that the present invention is directed to the enhanced transformation and regeneration of transformed embryogenic pine tissue in which the pine is of the genus *Pinus*, subgenus *Pinus*, which are the hard pines. *See* Paragraph 6 of the Exhibit 2 Connett Declaration. Dr. Connett-Porceddu also states that it was discovered that hard pines could be transformed and regenerated to produce transgenic hard pine plants using the disclosed and claimed method of the present application. *See* Paragraph 6 of the Exhibit 2 Connett Declaration. The present invention allowed for the first time *Agrobacterium*-transformation followed by regeneration of transgenic hard pine plants at a significant frequency. *See* Paragraph 6 of the Exhibit 2 Connett Declaration.

Dr. Connett-Porceddu states that the cited prior art (Levee et al.) discloses the transformation and regeneration of pine of the subgenus *Strobus* which the authors characterized as the first report

of the successful stable genetic transformation of a pine species. However, this prior art does not show the transformation and regeneration of pines of the subgenus *Pinus*, and a skilled artisan would not expect that the method for soft pines (subgenus *Strobus*) could be used or routinely modified for use with hard pines (subgenus *Pinus*). *See* Paragraph 7 of the Exhibit 2 Connett Declaration.

To support her latter statement, Dr. Connett-Porceddu states that it was known at the time of the present invention that there were differences between soft pines and hard pines as seen in transformation and regeneration methods for soft pines and hard pines, such that there were no expectation of success with respect to the transformation of hard or soft pines on the basis of the other. *See* Paragraph 8 of the Exhibit 2 Connett Declaration. Dr. Connett-Porceddu describes the differences between hard and soft pines in Paragraph 9 of the Exhibit 2 Connett Declaration, including their classification and different susceptibility to diseases and *Agrobacterium* infection. Additional differences between hard and soft pines (a) has been shown for somatic embryogenesis of hard and soft pines as shown by Klimaszewska et al. and (b) is well known in the art as shown by the Declaration Under Rule 132 of Dr. Micahel Becwar. *See* Paragraph 10 of the Exhibit 2 Connett Declaration.

Dr. Connett-Porceddu states that (a) there had been no reports of the regeneration of transgenic plants of hard pines (i.e., pines of the subgenus *Pinus*) prior to the present invention and (b) any reports at all concerning regeneration of transgenic hard pines demonstrated that regeneration was not achieved (e.g., Wenck et al., cited by the Examiner in this application). *See* Paragraph 11 of the Exhibit 2 Connett Declaration. Dr. Connett-Porceddu also states that it is noteworthy that the cited Levee et al. prior art did not discuss at all the regeneration of transgenic plants of hard pine which is the most economic species of conifers. *See* Paragraph 12 of the Exhibit 2 Connett Declaration. Dr. Connett-Porceddu also states that there have no reports of the application of the method of Levee et al. to the regeneration of transgenic hard pines and in fact, Levee himself has not continued use of the disclosed method for even soft pines. *See* Paragraph 12 of the Exhibit 2 Connett Declaration. Dr. Connett-Porceddu further states the assignee of the present application has

tried to use or modify the method described by Levee et al. for the regeneration of transgenic hard pine but has not been successful. *See* Paragraph 12 of the Exhibit 2 Connett Declaration.

Dr. Connett-Porceddu states that experiments had been underway at the assignee of the present application for more than 10 years to adapt systems for regeneration hard pines and for transforming and regenerating transformed hard pines. She states that somatic embryogenesis systems had been developed which worked well with hard pines, but not with transgenic hard pines. *See* Paragraph 13 of the Exhibit 2 Connett Declaration. The inability to adapt systems developed for transgenic soft pines to transgenic hard pines is further evidence of the differences between soft pines and hard pines and is evidence of no expectation of success in the art for using systems for transgenic soft pines for regenerating transgenic hard pines. *See* Paragraph 13 of the Exhibit 2 Connett Declaration. Since (a) a person of ordinary skill in the art knew that there were differences between soft pines (subgenus *Strobus*) and hard pines (subgenus *Pinus*) with respect to tissue culture, regeneration and transformation and (b) there was a lack of application of methods between the soft and hard pines, there was no expectation of success in the art for regenerating transgenic hard pines on the basis of a single report for the regeneration of transgenic soft pines. *See* Paragraph 14 of the Exhibit 2 Connett Declaration.

The Declaration Under Rule 132 of Michael R. Becwar (hereinafter the "Becwar Declaration") describes the nonobvious nature of the present invention over the art cited by the Examiner. Specifically, Dr. Becwar summarizes the invention claimed in the present application and states that it allowed for the first time the efficient regeneration of transgenic plants of the economically important pines of the subgenus *Pinus*, particularly the Southern yellow pines and hybrids thereof. *See* Paragraph 9 of the Becwar Declaration.

Dr. Becwar explains differences between hard and soft pines and expectations of skilled artisans with respect to working with these conifers based on his experience. *See* Paragraph 10 of the Becwar Declaration. Dr. Becwar states that he has worked with both soft pines, particularly *P. strobus*, and hard pines including *P. taeda*, and that his group was the first to report obtaining somatic embryogenic cultures for a soft pine. *See* Paragraph 10 of the Becwar Declaration. He also

states that although there are similarities in the stage of culture initiation and general appearance of embryogenic cultures of *P. strobus* and hard pines such as *P. taeda*, the similarities end there. *See* Paragraph 10 of the Becwar Declaration. He states that it is generally known and well accepted by those skilled in conifer somatic embryogenesis that what works for one group of pines will not work with another group of pines and provides examples to support his statement. *See* Paragraph 10 of the Becwar Declaration. Finally, he states that the knowledge and acceptance in the art that what works with one group of pines will not necessarily work with another group of pines has been his experience in his work with soft and hard pines. *See* Paragraph 10 of the Becwar Declaration.

Dr. Becwar discusses the teachings of the prior art cited by the Examiner. He states that Wenck et al. specifically teaches that genetically modified plants of Southern yellow pines had not been obtained. *See* Paragraph 11 of the Becwar Declaration. This result is opposite to the result that Wenck et al. achieved for Norway spruce, a different conifer. He further states that, in his opinion, Wenck et al., which only obtained transient transformation in loblolly pine (a Southern yellow pine) would not make obvious the methods taught in the present application. *See* Paragraph 12 of the Becwar Declaration. He states that since Wenck et al. did not teach a method resulting in stably transformed (transgenic) loblolly pine trees, it was a scientific exaggeration for the authors to include in the title "high efficiency" of both species. *See* Paragraph 12 of the Becwar Declaration. Dr. Becwar further states that the invention described in the present application provides methods that have commercial-scale applicability which separates the present invention from the published literature, including Wenck et al. *See* Paragraph 12 of the Becwar Declaration.

. Dr. Becwar states that Rutter et al. describes a method for regenerating pine plants from tissue culture in which PEG and ABA are used during a very distinct and specific stage of somatic embryogenesis, namely during the stage when cultures are induced to develop somatic embryos and to mature the embryos. *See* Paragraph 13 of the Becwar Declaration. He states that this stage is referred to as embryo maturation since the culture undergoes a morphological change from having relatively undifferentiated tissue with only very early stage embryos to tissue have more fully developed (mature) embryos. *See* Paragraph 13 of the Becwar Declaration. Dr. Becwar states that

this process is completely different than the use of ABA and/or PEG during the selection process for obtaining stable transgenic cell lines of the present application. *See* Paragraph 13 of the Becwar Declaration. He states that the physiological reason that ABA and/or PEG is applied in the embryo maturation case, such as disclosed by Rutter et al., is to induce the very early stage embryos to develop and mature. He also states that the ABA and/or PEG is not being used during the selection process of the present invention to induce development or maturation of embryos. He further states that it appears that the ABA and/or PEG in the present invention is stimulating the growth of transgenic tissue which is a different response than that of Rutter et al. *See* Paragraph 13 of the Becwar Declaration. Dr. Becwar states that there is nothing in Rutter et al. which suggests that ABA and/or PEG should be used in the selection process, particularly since Rutter et al. is directed to methods for somatic embryogenesis and is not directed to methods for obtaining transgenic plants. *See* Paragraph 13 of the Becwar Declaration. Dr. Becwar then concludes that it is his opinion that a skilled artisan would have no expectation of success for modifying the method of Wenck et al. as proposed by the Examiner to regenerate genetically modified plants of Southern yellow pines because Wenck et al. did not regenerate genetically modified plants from selected transgenic pine cells and Rutter et al. did not select transgenic pine cells and did not regenerate plants from selected transgenic pine cells. *See* Paragraph 13 of the Becwar Declaration.

It is also Dr. Becwar's opinion that a skilled artisan would have no expectation of success for modifying the method of Wenck et al. as proposed by the Examiner to regenerate genetically modified plants of Southern yellow pines because Wenck et al. did not regenerate genetically modified plants from selected transgenic pine cells and Levee et al. did not select transgenic pine cells of Southern yellow pines and did not regenerate plants from selected transgenic pine cells. *See* Paragraph 14 of the Becwar Declaration.

It is further Dr. Becwar's opinion that a skilled artisan would have no expectation of success for modifying the method of Wenck et al. as proposed by the Examiner to regenerate genetically modified plants of Southern yellow pines because Wenck et al. did not regenerate genetically modified plants from selected transgenic pine cells, Levee et al. did not select transgenic pine cells

of Southern yellow pines and did not regenerate plants from selected transgenic pine cells and Rutter et al. did not select transgenic pine cells and did not regenerate plants from selected transgenic pine cells. *See* Paragraphs 15-16 of the Becwar Declaration.

The Declaration Under Rule 132 of David S. Canavera (hereinafter the "Canavera Declaration") describes the long felt need in the art for the transformation and regeneration of transformed tissue of pines of the subgenus *Pinus*, i.e., hard pines. Specifically, Mr. Canavera states that (i) hard pines, particularly loblolly pine (*Pinus taeda)* and including *P. rigida* and *P. radiata* are the most commercially important pines and (ii) transformation followed by reliable regeneration of hard pines is more desirable than the transformation and regeneration of other conifers, such as white pines (subgenus *Strobus*) and spruces. The transformation of hard pines has turned out to be more difficult than for other conifers. *See* Paragraph 6 of the Canavera Declaration.

Mr. Canavera states that researchers have been attempting to transform hard pines with *Agrobacterium* followed by reliable regeneration since about 1988-1989. He also states that although *Agrobacterium* transformation of hard pines had been achieved and regeneration of hard pines had been achieved, regeneration of *Agrobacterium*-transformed hard pines had not been achieved despite considerable effort. *See* Paragraph 7 of the Canavera Declaration. These facts are corroborated by the references included as Exhibits 1 and 2 to the Canavera Declaration and the dearth of published reports on regenerated transformed hard pines. *See* Paragraph 7 of the Canavera Declaration. Thus, Mr. Canavera states that there was a long-felt need to develop (i) improved methods of *Agrobacterium* transformation of hard pines and improved selection of transformed tissue and (ii) methods to regenerate *Agrobacterium*-transformed hard pines. This long felt need was not satisfied by transformation of other conifers. *See* Paragraph 7 of the Canavera Declaration.

Mr. Canavera further states that the method for selection of transgenic Southern yellow pine tissue described and claimed in the present application and the method for hard pine transformation and regeneration described and claimed in companion application Serial No. 09/973,088 are the first methods that achieved reliable and efficient regeneration of transgenic hard pine plants. The reliable and efficient regeneration of transgenic hard pine results directly from the methods described and

claimed in these applications. *See* Paragraph 8 of the Canavera Declaration. Mr. Canavera also states that these methods are sufficiently robust to fill the long-felt need because the methods have been shown to be valid for a wide variety of genotypes of hard pines, a result which had not been achieved without these methods. *See* Paragraph 8 of the Canavera Declaration. Thus, Mr. Canavera concludes that the methods claimed in Serial Nos. 09/973,088 and 09/973,089 meet the long-felt need of providing regenerated *Agrobacterium*-transformed hard pines. *See* Paragraph 8 of the Canavera Declaration.

Finally, Mr. Canavera states that a method such as one used by the Canadian Forest Service (Levee et al.) that is not able to be used for multiple species did not meet the long felt need for the regeneration of transgenic hard pines, whereas the method described and claimed in Serial No. 09/973,088 does satisfy the long-held need. *See* Paragraph 9 of the Canavera Declaration. Mr. Canavera also states that a method that did not enable selection even from within elite families of loblolly pine would not meet the long felt need, whereas the method described and claimed in Serial No. 09/973,089 does satisfy the long-held need. *See* Paragraph 9 of the Canavera Declaration.

The Declaration Under Rule 132 of James E. Mann (hereinafter the "Mann Declaration") describes the commercial success of the present invention. Specifically, Dr. Mann states that hard pines, particularly loblolly pine (*P. taeda)* and including *P. rigida* and *P. radiata* are the most commercially important pines. *See* Paragraph 6 of the Mann Declaration. Dr. Mann also agrees with Mr. Canavera that (i) there was a long felt need for the transformation and regeneration of transformed tissues of pines of the subgenus *Pinus* (i.e., the hard pines) and (ii) that this long felt need was not satisfied by the transformation of other conifers, such as white pines and spruces. *See* Paragraph 7 of the Mann Declaration.

Dr. Mann also states that his employer, ArborGen, desired a robust, commercializable system for repeatable, reliable pine transformation followed by efficient selection and embryo development/formation. Dr. Mann states that ArborGen licensed the present application and companion application Serial No. 09/973,088, because suitable protocols for such a system did not exist elsewhere. *See* Paragraph 8 of the Mann Declaration. Dr. Mann further states that AborGen

is actively using the methods claimed in these applications and have transformed plants in field trials. *See* Paragraph 9 of the Mann Declaration. Finally, Dr. Mann states that AborGen has been approached by other researchers desiring to enter into deals with ArborGen so that AborGen would use the methods described in these licensed patent applications to prepare transgenic hard pine plants with genes of interest to these researchers. According to Dr. Mann, this fact is further evidence of the commercial value of the methods claimed in Serial Nos. 09/973,088 and 09/973,089. *See* Paragraph 10. Thus, Dr. Mann concludes that these facts demonstrate the commercial success of the methods claimed in these applications. *See* Paragraph 11 of the Mann Declaration.

It is submitted that the Rule 132 Declarations filed concurrently herewith establish that the present invention is not obvious from the teachings of Wenck et al., Rutter et al. and Levee et al. It is further submitted that the use of a differentiation agent as specified in the claims in a selection medium also containing a selection agent to select transgenic embryogenic pine cells of Southern yellow pines is not obvious from the teachings of Wenck et al., Rutter et al. and Levee et al. Thus, it is submitted that the claimed invention is not obvious from the teachings of Wenck et al., Rutter et al. and Levee et al. Withdrawal of this rejection is requested.

In summary, none of the cited references disclose the use of ABA, PEG or a gelling agent in the specified amount in a selection medium which is used for selecting transgenic embryogenic pine cells of the genus *Pinus* selected from the group consisting of Southern yellow pines and hybrids thereof. Also, none of the cited references disclose the regeneration of genetically modified plants of the genus *Pinus* selected from the group consisting of Southern yellow pines and hybrids thereof. Furthermore, the evidence presented in the prior art and in the Rule 132 Declarations demonstrate that there is no expectation of success in this art, and more particularly, there is no expectation that methods useful for one conifer can be used for another conifer. Thus, it is submitted that the combination of Wenck et al., Levee et al. and Rutter et al. does not render the claimed invention obvious. Withdrawal of this rejection is requested.

The Examiner provisionally rejected claims 1, 3, 31 and 33-37 under the judicially created doctrine of obviousness-type double patenting over claims 52-57 of copending U.S. application

Serial No. 09/973,088. Applicants submit herewith a Terminal Disclaimer to obviate this rejection. Withdrawal of this rejection is requested.

In view of the above amendments and remarks, in conjunction with the remarks made in the previous amendments, it is believed that the claims satisfy the requirements of the patent statutes and are patentable over the prior art. Reconsideration of the instant application and early notice of allowance are requested. The Examiner is invited to telephone the undersigned if it is deemed to expedite allowance of the application.

Respectfully submitted,

ROTHWELL, FIGG, ERNST & MANBECK, p.c.

By _____

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